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Chemical Nature of *Ganoderma lucidum* (Curtis) Karsten from Woodlands of Edo State, Nigeria.

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ABSTRACT
Samples of matured and naturally growing sporophores of *Ganoderma lucidum* (Curtis) Karst., an indigenous medicinal mushroom were collected from three separate local Government areas in Benin City, Edo State, Nigeria and analyzed for chemical substances. Alkaloids, saponins, flavonoids, were present in all the sporophores analyzed irrespective of their location while anthraquinone was absent in all. Sodium (Na) recorded the best values of between 0.924mg/g and 2.137mg/g while lead (Pb) had the least range value (0.017mg/g-0.021mg/g). The fruit bodies of *G. lucidum* from the University of Benin woodlands recorded the highest values for Na and calcium (Ca) respectively. The best protein, polysaccharide and lipid values recorded were 25.134%, 1.67% and 0.70% (dry weight per gram) of analyzed samples respectively. The spectrum of chemical constituents in the matured sporophores of local *G.lucidum* picked from the three sampled locations is fundamental to their uses as traditional remedies of diverse ailments.

KEYWORDS: Sporophores, *Ganoderma lucidum*, Chemicals, Alkaloids, traditional.

INTRODUCTION

Ganoderma lucidum (Curtis) Karst. is a polypore mushroom of the family Polyporaceae belonging to a group of fungi that are relatively benign when compared to their gilled “cousins”, some of which can be very poisonous. Polypore mushrooms have been the ancient “guardians” of the forest and forest peoples since prehistoric times (Wasser, 2002). The fruit body of *G. lucidum* is identified with a glossy reddish-orange to brownish-black colour. It has a definite stalk which is laterally or eccentrically attached to the cap. Stalkless (sessile) specimens have also been recorded in Nigeria and many parts of the world. *G. lucidum* is conk-like or kidney shaped with a woody texture, surface lacquered when moist, measuring 5-20cm in diameter and somewhat zoned (Arora, 1991). This mushroom which is one of the white rot fungi known to foresters often causes the root rot of aging and/or diseased trees, making them more easily susceptible to strong wind. The local names of *G. lucidum* differ dialectically across cultures and tribes. *G. lucidum* has common socio-cultural uses across Nigeria as traditional herb and/or as an ingredient in herbal preparations, spiritualism and mysticism, a pattern that was also reported in some places around the world (Wasser, 2005). Although, many polypores including *G. lucidum* are generally too tough to eat, rural people long ago discovered that a boiled tea from the fungus is health strengthening with anti-microbial and stimulatory potencies (Hobbs, 1995).

Documentary evidence on folk uses of indigenous mushrooms in Nigeria are limited and rudimentary compared to what obtains in China and Japan where mushrooms are exploited for over 4000 years as food and in the treatment of a wide range of human ailments i.e. hepatopathy, chronic hepatitis, nephritis, hypertension, arthritis, neurasthemia, insomnia, bronchitis, asthma and gastric ulcer (Akpaja *et al*., 2005). Hobbs (1995), Gao, (2002) and Wasser (2002) reported that *G. lucidum* contains a combination of potent enzymes, polysaccharides (antitumour, immuno-modulating), antioxidants and micronutrients in addition to water, organic and/or volatile soluble compounds such as amino acids, a small amount of protein and inorganic ions, steroids, triterpenes, lipids, alkaloids, glucoside, coumarin glycoside, volatile oil, riboflavin and organic acids including ganoderic acid (Ying *et al*., 1987). The polysaccharides and triterpenes isolated from *G. lucidum* are rated major active compounds with outstanding medicinal effects (Eo *et al*., 2000).

Information on the chemical nature of naturally occurring *Ganoderma* species reported in Nigeria i.e. *Ganoderma. colosum* (Fr.) C.F. Baker and *Ganoderma. boninense* Pat., is inchoate (Ofodile *et al*., 2005). Idu and Osemwegie (2007), Okhuoya *et al*. (2010) have reported the use of this mushroom as immuno-modulator, anti-allergy, antibiotic and anti-hypertensive, and in the treatment of anaemia, obesity and arthritis in Nigeria. Wasser (2005) reported that the global surge in the commerce of medicinal and edible mushrooms amount to $13 billion US dollars. This has challenged us to focus on indigenous wild utility mushrooms in Nigeria especially Edo State as part of ongoing chemical investigation. Literature reports on *Ganoderma* species concentrated more on the taxonomy, chemical composition, distributions, ethnomycology and cultivation of many American and Asian representatives with little or nothing on African varieties. This study was therefore aimed at evaluating the chemical constituents of *G. lucidum*, picked from across local Government areas of Edo State, Nigeria, in other to underpin its socio-cultural values and popularity as potent remedies of diverse ailments.

MATERIALS AND METHODS

Samples and chemicals

Fresh naturally occurring fruit bodies of *G.lucidum* used for the study were harvested with the use of a cutlass from felled decaying tree logs in randomly selected woodland systems located in Ogbeson village (A), Uselu (B) and University of Benin campus (C). These locations were distributed in Ikpoba-Okha, Egor and Ovia Local Government Areas of Edo State, Nigeria respectively. A total of 6 fruit bodies were randomly collected from 3 distanced (3-5km) woodland systems in a location. The glass and analytical grade chemicals use for this study were supplied by Biochemistry Department of the University of Benin while the study was carried out in the Mushroom Biology Lab of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria.

Chemical Analysis

In the lab, the fresh fruit bodies of *G. lucidum* from the field were oven dried at 55^0 C for 24 hours, manually cleaned with a small painting brush to remove all extraneous particles, sliced into smaller pieces with the use of a bread knife and further dried at 45^0 C for 30mins to ensure complete drying after which they were milled into powder using a mechanical blender, bagged and labeled samples A, B and C respectively. Two grams (2g) of the mushroom powder from each of the samples was analysed for metallic elements, alkaloids (anthraquinones, flavonoids, saponins and tannins) and ergastic contents such as protein, lipid and polysaccharides.

Metallic element analysis

The Fe, Ca, Na, Mg and Pb were analysed using HNO_3, H_2SO_4, $HClO_4$ wet-digestion reagents in ratio 10:5:10 method and determined by atomic absorption spectrophotometer (Varian model AA-1475) as outlined by Konuk *et al.* (2006).

Analysis of ergastic substances

Protein content was analyzed using the macro Kjeldahl method in which nitrogen contents was first determined and its value multiplied by 6.25 coefficient while the total lipid content was determined using Okalebo *et al*. (2002) Soxhelet extraction method. Twenty grams (20g) of the *G. lucidum* powder from each of the sampled locations (Ogbeson village, Uselu and University of Benin campus) were analyzed for polysaccharides using the Sephadex (G125 series) gel extraction and chromatographic methods (Lin *et al*., 2002)

Qualitative test of alkaloids

The method of Odebiyi and Sofowora (1978) was used in this test. Two grams (2g) of powdered sample from each of the locations was boiled in 30ml of 95% ethanol for 6 hours in a Soxhelet extractor. The extract was evaporated to dryness using a vacuum evaporator after which the residue was dissolved in 5ml of 1% HCl, shared into two equal parts respectively. Seven drops Mayer's reagent was added to one part while another seven drops of Dragendoff"s reagent was added to the other. Observed turbidity or precipitation with both reagents indicates the presence of alkaloids.

Saponins and flavonoids were analyzed using the qualitative method of Hertog *et al*. (1992) respectively while tannins was tested using drops of 10% $FeCl_3$ on the filtrate derived from a 500mg/10ml mixture of powdered sample and distilled water (Desphande *et al*., 1986). Anthraquinones was analysed by shaking 10ml benzene with one grams of the powdered samples respectively after which the filtrate from the mixture was treated with ten mililitre (10ml) of 10% NH_4OH fractionated using a separating funnel and observed for colour indicator in the ammonia layer (Odebiyi and Sofowora, 1978).

Statistical analysis

Each sample was analysed in triplicate and the values were then averaged. Data were assessed by the analysis of variance (ANOVA) as described by Snedecor and Cochran
(1987) and by Duncan-multiple range test with a probability $P \leq 0.05$.

RESULTS AND DISCUSSION

The results of the study showed that alkaloids and their chemical allies i.e. saponins, flavonoids and tannins were present in *G. lucidum* from the different locations while anthraquinone was absent (Table 1). These compounds in addition to both trace and major elements have been previously investigated in many socio-culturally valuable and edible mushrooms some of which include *Chlorophyllum molybditis*, *Lentinus subnudus*, *Pleurotus tuberregium*, *Psathyrella antroumbonata* and *Schizophyllum commune* by Alector (1995) and Alofe *et al*. (1996). Literature are however scarce on the chemical composition of many *Ganoderma* species in Nigeria compared to works done on other edible mushrooms (Ofodile *et al*., 2005; Osemwegie *et al*., 2006; Jonathan *et al*., 2008). The presence of alkaloids, flavonoids, saponins and tannins may be one of the reasons for the soaring popularity of *Ganoderma* in local healthcare provisions in many parts of the world especially in Nigeria (Osemwegie *et al*., 2006). The effect due to the absence of anthraquinone on the medicinal potency and immune-enhancing capability of this macrofungus is not fully understood despite reports of its presence in novelty plant material employed in phytomedicine (Odebiyi and Sofowora, 1978).

Table 1: Qualitative parameters of secondary metabolites and alkaloids

Sample	Alkaloids	Saponins	Flavonoids	Tannins	Anthraquinones
Ogbeson village (A)	+	+	+	+	-
Uselu (B)	+	+	+	+	-
University of Benin campus (C)	+	+	+	+	-

+ = present - = absent

G. lucidum from various locations in Edo State had the same range of protein (% dry weight) as those recorded in separate studies elsewhere (Mattila, 2001). *G. lucidum* collected from forests in Uselu, Egor LGA recorded the highest protein value (25.134%) while those from Ogbeson forests and University of Benin campus recorded 22.75% and 21.21% protein respectively (Table 2). The difference in protein content of the mushroom from the three (3) locations was not significant ($P \leq 0.05$). Fasidi and Kadiri (1992) also showed that *C. molybditis* and *P. tuberregium* in the South-Western part of Nigeria had protein content of 22.73 and 20.30% (dry weight) respectively. The marginal variation observed in protein contents of mushrooms is not fully understood but may be connected to differences in resource utilization/selectivity, the chemical dynamics and integrity of their macrohabitat. Although, the amino acids content of these samples was not analyzed, the value of the protein content is recognized according to Wasser (2005) to be an acceptable reflection of the amino acid value. The results from the study therefore confirmed that *G. lucidum* is fit for consumption but Osemwegie *et al*. (2006) remarked that its rejection was because of its glossy outlook and corky texture.

Table 2: Protein values (% dry weight) of *G. lucidum* from the different sampled locations

Sample	% per gram
Ogbeson village (A)	22.750
Uselu (B)	25.134
University of Benin campus (C)	21.218

Lipid values recorded during the study range from 0.6%-0.7% per gram of each of the sample analyzed respectively (Table 3). The difference in lipid values of the *G. lucidum* recorded from the three (3) locations was not significant ($P \leq 0.05$). This agreed with the work of Fasidi and Ekuere (1993) who in a separate study recorded values ranging between 0.20% - 1.02% for sclerotia of *P. tuberregium* cultivated on various waste materials.

Table 3: Lipid composition of *G. lucidum* samples (% dry weight).

Sample	Weight of Lipid	% Dry weight
Ogbeson village (A)	0.012	0.6
Uselu (B)	0.012	0.6
University of Benin campus (C)	0.014	0.7

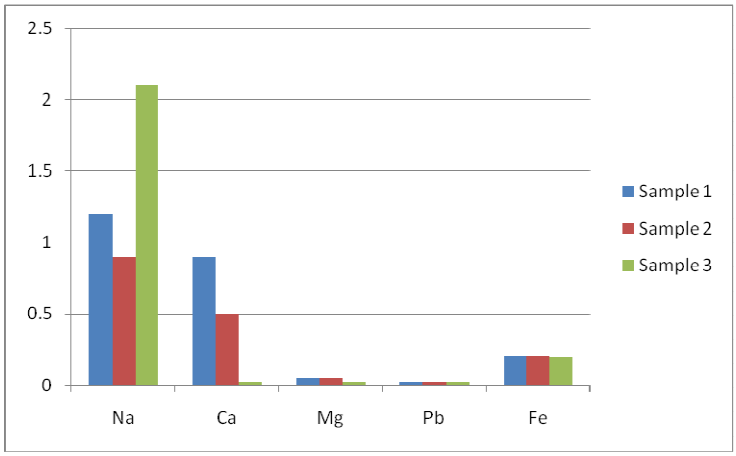
Polysaccharide values of 1.5 and 1.22% (dry weight) were recorded for fruit bodies of *G. lucidum* picked from forests in Ogbeson and University of Benin campus respectively while 1.67% (dry weight) was recorded for those picked in Uselu, Egor LGA of Edo State (Table 4). The difference in Polysaccharide content was also observed to be insignificant ($P \leq 0.05$). This agreed with Chang (1996) and Wang *et al.* (1996) who reported polysaccharide values of 1.38% for *G. lucidum* obtained from China and The Philipines respectively. The polysaccharides found in *G. lucidum* belongs to either the β-glucans groups which according to Chan *et al.* (2009) is responsible for the stimulation of many kinds of immune response and/or cells health in many animals and humans. Lentinan and Krestin were some of the β-glucan group that was reported by Wasser (2002) as proceeding through clinical trials in the treatment of cancers and other diseases.

Table 4: Polysaccharide values of *G. lucidum* samples (% dry weight)

Sample	Absorbance(nm)	Conc (µg/ml)	% per gram
Ogbeson village (A)	0.170	75.00	1.50
Uselu (B)	0.189	83.50	1.67
University of Benin campus (C)	0.138	61.00	1.22

The study also showed that non-toxic elements such as Na recorded the highest value of 2.10 mg/g dry weight followed by Ca (0.90mg/g) and Fe (0.25mg/g) respectively while Mg and Pb recorded the lowest values of 0.05mg/g and 0.02mg/g respectively. Fruit bodies of the sample collected from the University of Benin recorded the highest value for Na and Ca (Fig. 1). The difference in Na and Ca content was observed to be significant ($P \leq 0.05$) while that of Mg, Pb and Fe was not significant ($P \leq 0.05$). Analysis of fruit bodies from other locations recorded values for Ca which varied from 0.502-1.162mg/g (dry weight) as compared with that from University of Benin with 1.162mg/g dry weight value. This concurred with quantitative record of Konuk *et al.* (2006) and Olumuyiwa *et al.* (2008) on mineral composition of edible mushrooms from Turkey and Nigeria respectively but marginal differences were observed which may be as a result of variation in environmental and vegetation status, and level of anthropogenic activities. The values recorded for Ca during this study however concord with the work of Kadiri and Fasidi (1992) which reported 0.642mg/g and 1.25mg/g in *L. subnudus* and *P. tuberregium* respectively (Okhuoya and Ajerio, 1988). However, vast scientific literature supported interspecific and intraspecific variations in the elemental and chemical compositions of many utility mushrooms with philosophical rather than data-supported scientific conjectures provided as reasons (Isiloglu *et al*., 2001; Sanme *et al*., 2003). The

employment of fungal biomass, molecular and radioactive labeling techniques may provide broader insights to the influence of environmental variables and synecological characteristic on the mechanism of resource utilization, selectivity and assimilation by fungi differing in substrate preference or fungus from similar and different ecozones.



Mineral elements vs concentration (mg/g dry wgt)

Fig 1: The mineral elements of *G. lucidum* from different sampled locations (A=1; B=2; C=3)
(A) Ogbeson village (B) Uselu (C) University of Benin campus

Sodium (Na) recorded a value of 1.200mg/g for *G. lucidum* collected from Ogbeson forests, 0.924mg/g and 2.137mg/g for those from the forests of Uselu and University of Benin respectively, fell within the range reported by Kadiri and Fasidi (1992) for *Termitomyces robustus* (3.926mg/g), *L. subnudus* (2.298mg/g), Kadiri and Fasidi (1992) for *P. tuberregium* (2.298mg/g), and Mattila (2001) for *Lentinus edodes* (1.14mg/g). Vetter (2003) however remarked that the relatively consistent Na level of most edible mushrooms (0.10mg/g - 9.5mg/g) studied is of fundamental nutritional and medicinal benefit to the consumer, especially people with high blood pressure.

A lower range of values (0.021 and 0.034) was recorded for Magnesium (Mg) in *G. lucidum* samples collected from the three locations (A, B and C). This range is in consonance with the value of 0.037mg/g reported in an earlier work done by Wang *et al*., (1996) but varied from values recorded by Kadiri and Fasidi (1992) for *C. molybditis* (1.868mg/g) and *P. tuberregium* (1.484mg/g), and Mattila (2001) for *Agaricus bisporus* (0.10mg/g) and *L. edodes* (0.13mg/g). The low level of Mg recorded for *G. lucidum* may not be unconnected to the chemical nature of the substrates which were mostly wood-based. This according to Okhuoya and Ajerio, (1988) is due to the relatively low level of Mg in soils than calcium and the decrease in uptake of magnesium due to the high concentration of calcium in living tree plants. The level of Iron (Fe) also recorded for this fungus which ranged between 0.156mg/g and 0.176mg/g also concurred with the findings of Wang *et al*., (1996). Further studies are however necessary for a proper understanding of the origin and dynamics of chemical elemental flux in mushrooms substrates (nutrient base) *vis-á-vis* their absorption and assimilation.

The low value observed for Lead (Pb) from the *G. lucidum* samples from Ogbeson village, Uselu and University of Benin campus woodlands ranged from 0.017mg/g - 0.021mg/g. This was corroborated by Ziegler (2001) and Olumuyiwa *et al*. (2008) who categorized Pb as one of the trace toxic elements identified in wild edible mushrooms from Nigeria. The presence of Pb and its implication on the edible and medicinal utility of wild mushrooms requires further study even though the knowledge of the source of the

element is common. The relatively higher value of 0.021mg/g recorded for samples of *G. lucidum* from the University of Benin may be due to increased industrial and vehicular activities and/or emissions within the vicinity of the woodland from which the mushroom was picked (Isilogu *et al*., 2001).

It is obvious from this study that, *G. lucidum*, widely acclaimed for its medicinal properties, contains essential mineral nutrients which are of immense health benefit as antioxidative, vitamins, anti-inflammatory and antimicrobial in human and animals (Ofodile *et al*., 2005; Ogbe *et al*., 2008). Wasser (2002) reported that this fungus can boost the human protein level and immune systems through the range of polysaccharides that were anti-tumour (triterpenoids) in nature. Our body structure is made up mainly of bone and cartilage, so we need these minerals especially calcium for our body growth. The shiny/glossy appearance is as a result of the presence of tannin.

G. lucidum is consumed as tea by cutting whole mushroom into small pieces, sundried or oven dry and simmer in a cup of hot water. It can also be consumed in refined form as tablet. Though the fungus is a fundamental health food, its very woody nature was the reason for its inedibility either in pickled, fried, cooked or raw form. This study has therefore introduced preliminary information on the chemical nature of *Ganoderma lucidum* indigenous to Nigeria. It has also provided comparative base for quantitative and qualitative chemical data of Nigerian *Ganoderma* species with widely published varieties from Asia, America and Europe.

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